M. Pinkerton

AMRL-TR-68-179
AD0715973

EFFECTS OF EXPOSURES TO 100% OXYGEN ON CELLULAR METABOLISM

CLARE C. JOHNSTON
ROGER N. PETERSEN
HAROLD F. LEAHY
IIT Research Institute

AUGUST 1969

AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Federal Government agencies and their contractors registered with Defense Documentation Center (DDC) should direct requests for copies of this report to:

DDC
Cameron Station
Alexandria, Virginia 22314

Non-DDC users may purchase copies of this report from:

Chief, Storage and Dissemination Section
Clearinghouse for Federal Scientific & Technical Information (CFSTI)
Sills Building
5285 Port Royal Road
Springfield, Virginia 22151

Organizations and individuals receiving reports via the Aerospace Medical Research Laboratories' automatic mailing lists should submit the addressograph plate stamp on the report envelope or refer to the code number when corresponding about change of address or cancellation.

Do not return this copy. Retain or destroy.

The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 89–544, "Laboratory Animal Welfare Act," August 24, 1967.

100 - October 1969 - CO455 - 93-2056

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
IIT Research Institute 10 West 35 Street Chicago, Illinois 60616	UNCLASSIFIED 2b. GROUP N/A

3. REPORT TITLE

EFFECTS OF EXPOSURES TO 100% OXYGEN ON CELLULAR METABOLISM

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report, January 1968-September 1968

5. AUTHOR(S) (First name, middle initial, last name)

Clare C. Johnston
Roger N. Petersen
Harold F. Leahy

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
August 1969	39	13

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
F33615-68-C-1270	
b. PROJECT NO. 7163	IITRI-L6048-4
c. Task No. 716300	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d. Work Unit No. 716300005	AMRL-TR-68-179

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Aerospace Medical Research Laboratory, Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, CH 45433

13. ABSTRACT

Studies have been made of the biochemical toxicology of oxygen atmospheres. These studies are divided into two parts: (a) effects of pure oxygen atmospheres at ambient pressure on the cellular bioenergetics of monkey (Macaca mulatta) liver and kidney tissue, and (b) effects of pure oxygen atmospheres at approximately one-third atmospheric pressure on rat liver tissues. Monkeys were exposed for 4, 7, and 12 days to 100% oxygen at 748 mm Hg pressure in the Thomas dome. Surviving animals (3, 3, and 2) were compared with 5 normal ambient controls. Allowing for variations in animals there appeared to be a trend to diminished kidney ATP levels and to lowered P/O values obtained with liver or kidney mitochondria by the Warburg technique using succinate substrate. Isolated mitochondria from exposed animals showed respiratory control. Rats were exposed for 1, 4, 7, or 14 days to 100% oxygen at 257 mm Hg pressure in the Thomas dome. Experiments also were run in which 14-day exposed rats were returned to ambient air 1 and 48 hours prior to sacrifice. Experimental animals were paired with ambient controls. Elevations in tissue ATP concentrations were observed after 1, 4, and 7-day exposures; there was no significant elevation at 14 days. There was some indication of elevated Q(O/N) values in states 4 and 3 with isolated mitochondria from exposed animals using succinate substrate. Average respiratory control values were also elevated.

DD FORM 1 NOV 65 1473

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Oxygen Toxicity						
100% Oxygen						
Cellular Metabolism						
Blood Gases						
Kidney Function						
Monkeys						
Rats						

FOREWORD

This study was initiated by the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio 45433. The research was conducted at the Aerospace Medical Research Laboratories by the IIT Research Institute (IITRI) under Air Force contract F33615-68-C-1270. Clare C. Johnston, Ph.D., was the principal investigator for IITRI. Miss Marilyn E. George of the Toxicology Branch, Toxic Hazards Division, was the contract monitor for the Aerospace Medical Research Laboratories. The work was performed in support of project 7163, "Research on Biomechanisms and Metabolism." The research performed herein was started in January 1968 and was completed in September 1968.

Surgical removal of monkey tissues in the Thomas Domes was accomplished by Captain R. P. Bradbury and Major V. L. Carter, Jr. of the Toxicology Branch.

Valuable assistance was provided by Mr. J. P. F. Murphy of the Toxicology Branch. Contributing personnel at IITRI included Willis H. Riesen, Ph.D. and Ervin J. Hawrylewicz, Ph.D.

This report is cataloged by IITRI as Report No. IITRI-L6048-4.

This technical report has been reviewed and is approved.

C. H. KRATOCHVIL, Colonel, USAF, MC
Commander
Aerospace Medical Research Laboratory

TABLE OF CONTENTS

Section

I.	Introduction	1
II.	Methods and Materials	1
	Monkeys at 748 mm Hg Pressure	1
	Rats at 257 mm Hg Pressure	5
III.	Discussion	9
	Experiments Using Monkeys at 748 mm Hg Pressure	9
	Experiments Using Rats at 257 mm Hg Pressure	19
References		39

Appendix

Statistical Analysis of Data Obtained in Experiments Using Monkeys at 748 mm Hg Pressure	33

LIST OF TABLES

Table		Page
I	Survival Ratios for Monkeys after Various Length Exposures to 100% Oxygen at 748 mm Hg Pressure	2
II	Monkeys Used in the Study of the Effect of Various Length Exposures to 100% Oxygen at 748 mm Hg Pressure on Oxidative Phosphorylation in Liver and Kidney Tissues	3
III	Weights of Rats used in Exposures to 100% Oxygen at 257 mm Hg Pressure	6
IV	Terminal Arterial Blood P_{O_a} and P_{CO_a} Values of Monkeys Exposed to 100% Oxygen at 748 mm Hg	8
V	Tissue ATP Concentrations of Livers and Kidneys from Monkeys Exposed to 100% Oxygen at 748 mm Hg	11
VI	Q(O/N) Values of Liver and Kidney Mitochondria from Monkeys Exposed to 100% Oxygen at 748 mm Hg	13
VII	P/O Ratios of Liver and Kidney Mitochondria from Monkeys Exposed to 100% Oxygen at 748 mm Hg	14
VIII	Inorganic Phosphate Esterified per mg Mitochondrial Protein Nitrogen (at 30 C using α-Ketoglutarate or Succinate Substrates) by Liver and Kidney Mitochondria from Monkeys Exposed to 100% Oxygen at 748 mm Hg	15
IX	Q(O/N) in State 4 of Liver and Kidney Mitochondria from Monkeys Exposed to 100% Oxygen at 748 mm Hg	16
X	Q(O/N) in State 3 of Liver and Kidney Mitochondria from Monkeys Exposed to 100% Oxygen at 748 mm Hg	17
XI	ADP/O Ratios (Obtained at 25 C using the Clark Oxygen Electrode with α-Ketoglutarate or Succinate Substrates) of Liver and Kidney Mitochondria from Monkeys Exposed to 100% Oxygen at 748 mm Hg	18
XII	RC Values (Obtained at 25 C using a Clark Oxygen Electrode with α-Ketoglutarate or Succinate Substrates) of Liver and Kidney Mitochondria from Monkeys Exposed to 100% Oxygen at 748 mm Hg	20

LIST OF TABLES (cont.)

Table		Page
XIII	Tissue ATP Concentrations of Livers from Rats After Various Exposures to 100% Oxygen at 257 mm Hg	22
XIV	Q(O/N) in State 4 Using α-Ketoglutarate Substrate with Liver Mitochondria from Rats after Various Exposures to 100% Oxygen at 257 mm Hg	23
XV	Q(O/N) in State 4 Using Succinate Substrate of Liver Mitochondria from Rats After Various Exposures to 100% Oxygen at 257 mm Hg	24
XVI	Q(O/N) in State 3 Using α-Ketoglutarate Substrate of Liver Mitochondria from Rats After Various Exposures to 100% Oxygen at 257 mm Hg	25
XVII	Q(O/N) in State 3 Using Succinate Substrate of Liver Mitochondria from Rats after Various Exposures to 100% Oxygen at 257 mm Hg	26
XVIII	ADP/O Ratios Obtained Using α-Ketoglutarate Substrate of Liver Mitochondria from Rats After Various Exposures to 100% Oxygen at 257 mm Hg	27
XIX	ADP/O Ratios Obtained Using Succinate Substrate of Liver Mitochondria from Rats After Various Exposures to 100% Oxygen at 257 mm Hg	28
XX	RC Values Obtained Using α-Ketoglutarate Substrate of Liver Mitochondria from Rats After Various Exposures to 100% Oxygen at 257 mm Hg	29
XXI	RC Values Obtained Using Succinate Substrate of Liver Mitochondria from Rats After Various Exposures to 100% Oxygen at 257 mm Hg	30
XXII	Regression Equations: $y = c + b_1x + b_2x^2 + b_3x^3$, for Those Parameters (Discussed in the Report Text) for Which F Values of the Regression Coefficients Exceeded 3.0	37
XXIII	F Values of Regression Coefficients	38
XXIV	Standard Error of Regression Coefficients	39
XXV	Multiple Correlation Coefficients, F Values and Associated Significance Levels for Regression Equations	41

SECTION I

INTRODUCTION

The production and use of artificial atmospheres has greatly increased the need for research on the metabolism and toxicology of gaseous materials. Favorable tissue concentrations of molecular oxygen are critically balanced between deficiency and toxicity; metabolic reactions requiring oxygen must proceed but non-physiologic oxidations of sensitive chemical moieties must be avoided (Haugaard, 1968). The experimental research on effects on cellular bioenergetics of exposure to pure oxygen discussed in this report consists of two parts: (1) exposures at 748 mm Hg pressure for 4, 7, or 12 days, using monkeys (Macaca mulatta), and (2) exposures at 257 mm Hg pressure for 1, 4, 7, or 14 days, using rats. In addition, recovery experiments were conducted in the 257 mm Hg exposure study in which 14-day exposed animals were returned to ambient air 1 and 48 hours prior to sacrifice. Animal exposures were carried out in Thomas domes (Thomas, 1965). Using tissues obtained from exposed and ambient control monkeys, liver and kidney tissue ATP levels were determined and liver and kidney mitochondria were prepared. The oxidative and phosphorylative properties of the mitochondrial preparations were studied by both Warburg and oxygen-electrode techniques using α-ketoglutarate and succinate substrates. In the experiments involving rats, liver tissues from exposed and ambient controls were used to determine ATP levels and to prepare isolated mitochondria which were then studied using the oxygen electrode with α-ketoglutarate and succinate substrates. Data obtained indicate effects of oxygen on liver and kidney metabolism at one atmosphere and, also, upon rat liver metabolism at one-third atmosphere.

SECTION II

METHODS AND MATERIALS

MONKEYS AT 748 MM HG PRESSURE

The operation of the Thomas domes has been previously described. According to the information provided by the Aerospace Medical Research Laboratories, the average dome operating conditions were as follows:

Pressure:	748 mm Hg (Range, 700 to 750 mm Hg)
Temperature:	77.6 F (Range, 74 to 81 F)
Humidity:	59% RH (Range, 42 to 69% RH)
Carbon Dioxide Content:	0.095% (Range, 0.00 to 0.44%)
Air Flow Rate:	20 ft^3/min (Range, 18 to 35 ft^3/min)

Exposed and ambient control monkeys (Macaca mulatta) 2½ - 3½ years old were fed Purina Monkey Chow once a day and water ad libitum. Ambient control monkeys were maintained in the vivarium. Since the biochemical investigations were carried out on surviving animals, Table I is provided to indicate the survival ratios in the 100% oxygen atmosphere.

Table I

SURVIVAL RATIOS FOR MONKEYS
AFTER VARIOUS LENGTH EXPOSURES TO 100% OXYGEN
AT 748 MM HG PRESSURE*

(Data provided by
Aerospace Medical Research Laboratories)

Exposure time, days	Survival Ratio	
	No. Exposures**	Percent Surviving Exposure
4	31/32	97
7	17/24	71
12	5/17	29

*Numbers include all monkeys exposed during the experimental period, whether or not used by IITRI personnel.

**Numbers of animals, including sacrificed, surviving N days divided by number of animals dying of exposure on or before the Nth day plus number of animals, including sacrificed, surviving N days.

Each experiment was run on a separate day and included tissue from only one animal. The experiments usually commenced at about the same time each day. Animals were intravenously anesthetized "to effect" by i.v. injection of approximately 30 mg of Nembutal(R) per kg body weight. Arterial blood samples were taken and then kidney and liver tissues were obtained. Exposed animals remained in the dome during these operations. A description of the animals used is given in Table II.

Table II

MONKEYS USED IN THE STUDY OF THE EFFECT OF VARIOUS LENGTH EXPOSURES TO 100% OXYGEN AT 748 MM HG PRESSURE ON OXIDATIVE PHOSPHORYLATION IN LIVER AND KIDNEY TISSUES

(Data provided by Aerospace Medical Research Laboratories)

Number	Sex	Weight, kg
Ambient Controls		
D-85	F	3.64
D-91	F	4.1
D-95	F	4.1
D-96	M	-
D-97	F	3.7
E-39	F	-
4-Day Exposures		
D-63	F	3.64
D-98	M	3.6
E-27	F	2.9
7-Day Exposures		
D-07	F	4.5
E-31	F	3.41
E-35	F	3.6
E-37	F	4.15
12-Day Exposures		
D-77	F	4.55
E-04	M	4.0

Liver and kidney tissues used for determination of ATP content were frozen in situ using quick-freezing tongs (Riesen, 1967). The frozen tissue samples were then immersed in liquid nitrogen contained in a Dewar Flask for transport from the dome area to the laboratory where they were kept on dry ice in the deep freeze prior to analysis by the procedure used previously. Approximately 4.5 g of tissue to be used for the isolation of mitochondria was quickly excised, dropped into ice-cold 0.25 M sucrose, and minced. Samples were kept ice-cold and transported back to the laboratory in an ice bucket.

Mitochondria were prepared essentially by the method of Weinbach (1961) incorporating some modifications of Nelson et al. (1967). Approximately 4.5 g of chilled, diced tissue was homogenized for 30 sec in ice-cold 0.25 M sucrose (30% w/v) by means of an Ace tissue grinder having a glass-reinforced Teflon pestle (0.006 - 0.009 inch clearance). The homogenate was then diluted to a 10% w/v suspension with 0.25 M sucrose and centrifuged at 600 x g for 10 min in an International High-Speed Refrigerated

Centrifuge at approximately 0 C. The resulting supernatant was decanted and centrifuged at 8,500 x g for 10 min. The pellet washing step was repeated, and the twice-washed pellet was finally suspended in 0.25 M sucrose (1 ml/g original tissue wet weight). The time lapse from incision to remove tissue at the dome to the start of incubations of isolated mitochondria was approximately 90 min. Liver and kidney mitochondria were prepared at the same time and incubations of mitochondria were carried out by both Warburg respirometer and Clark oxygen-electrode techniques immediately following isolation.

The Warburg method used has been described in detail previously (Riesen, 1966). A Gilson Medical Electronics (GME) Oxygraph and a Clark Oxygen electrode, Yellow Springs Instrument Co., Inc. (YSI) were used in the oxygen-electrode method. A Haake Constant Temperature Circulating Pump was used to maintain close control of the incubation cell temperature of 25 C. Glycylglycine (Calbiochem A. grade) buffer (1.3 ml), pH 7.4, was added to the cell followed in order by 0.1 ml additions of mitochondrial suspension, phosphate and substrate. Substrates used were α-ketoglutarate (Sigma Chemical Co.) or succinate (Sigma Chemical Co.). The composition of the incubation medium was 120 μmoles KCl, 20 μmoles glycylglycine, 8 μmoles $MgCl_2$, 5 μmoles orthophosphate, and 10 μmole substrate/ml.

Mitochondria incubated in the above system in the presence of excess substrate, orthophosphate and oxygen are defined to be in state 4 (Chance, 1956). After a short time in this state, 6 μl of ADP (Sigma Chemical Co., sodium salt adjusted to pH 7.4 with potassium hydroxide) was added by means of Hamilton syringe (0.16 μmoles/ml incubation medium). Mitochondria incubated under these conditions, i.e. in the presence of excess phosphate acceptor, ADP, are defined to be in state 3. Uptake of dissolved oxygen from the medium by mitochondria in states 4 and 3 was recorded on the GME Oxygraph chart. If oxidation of substrate by the mitochondria is tightly coupled to phosphorylation of ADP, the rate of substrate oxidation in State 3 will be appreciably greater than that in state 4. The ratio of the rate in state 3 to that in state 4 is defined as the Respiratory Control (RC) of the mitochondria. The RC of damaged mitochondria decreases and becomes 1.0 when there is no stimulation of oxidation in the presence of excess phosphate acceptor.

Once the added ADP has all been phosphorylated, the rate of oxygen-uptake by the mitochondria should return to nearly the same state 4 rate originally observed. A change from state 4 to state 3 incubation conditions by addition of ADP represents one cycle. These cycles are repeatable by additions of more ADP. Oxygen uptake rates obtained in state 4 and 3 on the second and third cycles were used. RC values were calculated from the ratio of the rate of oxygen uptake in state 3 to the rate of the immediately preceeding state 4. The oxygen content of the medium was calculated by comparing Oxygraph chart

readings with the oxygen electrode in medium to readings with the electrode in 0.1 N NaCl solution of known oxygen content, at the given atmospheric pressure. The concentration of the ADP solution was determined spectrophotometrically (a_M = 15.4 x 10^3 at 259 mμ, pH 7.0). The ADP/O ratio was determined by dividing the amount of added ADP by the total oxygen consumed during the resulting state 3 oxidation period. After incubation of one aliquot of mitochondrial suspension through several cycles, the cell was cleaned and another incubation started.

The order in which incubations were carried out was (1) liver mitochondria using succinate substrate, (2) liver mitochondria using α-ketoglutarate, (3) kidney using succinate, and (4) kidney using α-ketoglutarate. Another set of duplicate incubations was carried out in the same order. Values presented in the tables represent the averages of the duplicate results obtained during the second and third cycles.

Aliquots of the original mitochondrial suspensions were used for determination of protein concentration by the biuret method. Nitrogen (mg/ml) was calculated from the protein analysis using the factor 0.16.

RATS AT 257 MM HG PRESSURE

Exposures were carried out in the Thomas dome. According to the information provided by the Aerospace Medical Research Laboratories, the average dome operating conditions were as follows:

Pressure:	257 mm Hg (Range, 252 to 260 mm Hg)
Temperature:	77.5 F (Range, 71 to 86 F)
Humidity:	52.5% RH (Range, 15 to 74% RH)
Carbon Dioxide Content:	0.05% (Range, 0.00 to 0.42%)
Air Flow Rate:	20 ft^3/min (Range, no change)

Exposed and ambient control male Sprague Dawley rats were fed Purina(R) Laboratory Chow and water ad libitum. The weights of the animals are shown in Table III.

To minimize effects of slight daily variations which may occur in the preparative procedures and analyses, an ambient control animal and an exposed animal were carried through the procedures and analyses concurrently. Exposed rats were anesthetized "to effect" by i.p. injection of 6.5 mg/100 g of Nembutal.(R) At the same time, ambient control animals were anesthetized outside the dome in ambient air. Liver tissue samples from both exposed and ambient control rats were obtained for ATP analysis and for isolation of mitochondria by the procedures used for monkeys.

Table III

WEIGHTS OF RATS USED IN EXPOSURES TO 100% OXYGEN AT 257 MM HG PRESSURE

(Data Provided by Aerospace Medical Research Laboratories)

Animal No.	Weight, g	
	Exposed Animal	Paired Ambient Control
1-Day Exposures		
1	153	150
2	175	172
3	218	228
4	241	295
5	251	280
6	182	195
7 to 11*	180	158
	181	182
	198	156
	201	187
	224	190
4-Day Exposures		
1	196	202
2	174	168
3	266	292
4	256	303
5	245	245
6	206	177
7 to 12*	222	275
	256	245
	242	244
	237	246
	256	250
	233	264
7-Day Exposures		
1	210	160
2	176	178
3	318	320
4	330	320
5	133	355
6	206	248
7	262	296
8 to 13*	305	270
	304	268
	274	275
	310	206
	302	184
	256	244

Table III (continued)

Animal No.	Weight, g: Exposed Animal	Weight, g: Paired Ambient Control
14-Day Exposures		
1	261	230
2	335	322
3	284	272
4	257	160
5	271	268
6 to 10*	210	198
	214	238
	215	252
	225	246
	248	252
14 Days plus 1 hr recovery		
1	267	273
2	240	220
3	250	265
4	262	225
5	300	334
6	355	360
14 Days plus 48 hr recovery		
1	292	302
2	198	265
3	212	270
4	358	348
5	298	361

*Weights given for the animals used, but animals not paired in order that weights are listed.

Table IV

TERMINAL ARTERIAL BLOOD P_{O_2} AND P_{CO_2} VALUES OF MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

(Data provided by Aerospace Medical Research Laboratories)

Monkey No.	Arterial Blood Values, mm Hg P_{O_2}	P_{CO_2}
Ambient Controls		
D-85	85	29.5
D-91	90.5	25.5
D-95	92	22.5
D-96	-	-
D-97	98	29.4
E-39	-	-
4-Day Exposures		
D-63	355	35
D-98	-	-
E-27	535	39.5
7-Day Exposures		
D-07*	103	70.5
E-31	302	38
E-35	138	30
E-37	50	43.5
12-Day Exposures		
D-77	355	33.5
E-04	48	40.5

* Animal died 30 minutes after blood sample was drawn under Nembutal(R) anesthesia.

Mitochondria were prepared concurrently from the exposed and ambient control rat livers, essentially as described for the experiments involving monkeys except that the 1.2 g of tissue was homogenized directly in a 10% w/v suspension of 0.25 M sucrose prior to the initial centrifugation. In order to avoid bias resulting from consistent time differences between isolation of mitochondria and incubation in the GME Oxygraph cell, mitochondria from exposed and ambient controls were generally incubated in opposite order on successive days. Values presented in the tables represent the averages of duplicate determinations during the second and third cycles. Aliquots of the original mitochondrial suspensions were used to determine mitochondrial nitrogen concentration per ml suspension using the biuret method for protein and a conversion factor of 0.16.

SECTION III

DISCUSSION

EXPERIMENTS USING MONKEYS AT 748 MM HG PRESSURE

The terminal arterial blood P_{O_2} and P_{CO_2} levels of the monkeys used in this study are shown in Table IV.

The arterial P_{O_2} levels of the monkeys used in this study were probably all elevated at four days; thereafter, considerable individual variations are seen. These variations are reflections of the different responses in the lungs to the high oxygen insult. These responses include alveolar edema, which may typically reach a maximum at about 4 to 7 days and then resolve, and proliferation of interstitium and alveolar epithelium which may occur on about the 5th day and advance with duration of exposure (Robinson, 1967). Efficiency of oxygen diffusion through the alveoli depends on the degree of congestion and proliferation that has occurred. Hence, the P_{O_2} carried to the livers and kidneys of these surviving animals by the blood was apparently very high for at least the first 4 days, after which time the P_{O_2} levels in some animals may have remained elevated while in others they decreased to normal and subnormal levels. The length of time that liver and kidney mitochondria were exposed to certain oxygen tensions is therefore not simply related to the duration of animal exposure.

Striking changes in hepatic mitochondria have been noted in rats exposed to oxygen at 760 mm Hg for 1 day, to 258 mm Hg for 1 to 14 days, and to 2280 mm Hg for 3 hr (Schaffner, 1965). Disappearance of cristae from renal mitochondria of dogs and monkeys exposed to 258 mm Hg for 14 and 90 days has been observed (Mautner, 1966). Morphological changes in renal and hepatic mitochondria suggest that the essential energy-producing systems contained in these organelles may also be affected. Initial studies on the oxidative properties of monkey renal and hepatic mitochondria following animal exposures to 100% oxygen at ambient pressure for 6 hr and 2, 4, 7 and 12 days have been reported (Riesen, 1967).

E-39 and D-96 were the first animals studied and the experimental procedures may have proceeded somewhat slower with these animals. The results from the data have been summarized and tabulated in Tables V through XII. Certain trends appear in these results, although there were not sufficient animals included in the experiment to permit valid statistical determinations of significance. Samples of tissue were taken from these animals and histological studies were performed. The results of these studies will be reported separately,* and they should greatly facilitate the interpretation of the data reported here. For convenience, a summary of some of the pathology results is presented.

The ambient control animals: monkey D-91, D-95 and D-96 were in good condition. The lungs of monkey D-85 were not entirely normal and there were scattered alveolar exudates; the kidneys of this animal showed mild but diffuse foamy, granular eosinophilic material in the tubules. Monkey D-97 had a borderline exudate in the lungs. No information on monkey E-39 is available. For the exposed animals: the 4-day exposed monkeys all exhibited mild congestion of the lungs. There was moderate peribronchiolar inflammation and mild focal alveolar edema. The lungs of the 7-day exposed animals showed marked septal thickening consisting of fibrinoblasts, collagen and fibrin. There was a marked degree of alveolar edema and exudate with consolidated fibrinocellular material. The two 12-day exposed monkeys exhibited markedly different degrees of lung involvement. Monkey D-77 had only minor edema and exudate with moderate thickening of septa. There was no diffusion problem with this animal. Monkey E-04 showed extreme diffuse organized alveolar exudate and extreme septal thickening (fibrinocellular). There was marked diffuse emphysema. This animal had a major diffusion problem. The kidneys of all exposed animals showed pathological changes, especially the 4- and 7-day exposed animals.

Table V shows the tissue ATP concentrations. In general, the ATP levels appeared to decrease as the duration of exposure was prolonged. The decline was not as large nor as uniform in the liver as in the kidney. However, one control monkey showed the lowest kidney tissue ATP concentration of any of the animals (D-85, note pathology description in preceeding paragraph). A vigorous animal, E-04, maintained a normal liver ATP level after 12 days exposure, but the kidney ATP level of this monkey was also low.

---

*George, Marilyn E. et al., 1968. Toxic Effects in Monkeys Exposed to 100% Oxygen at Ambient Pressure. AMRL-TR-68-178, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio (In preparation).

Table V

TISSUE ATP CONCENTRATIONS OF LIVERS AND KIDNEYS FROM MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

Monkey No.	ATP, μmoles/g Liver	Kidney
Ambient Controls		
D-85	2.6	0.7
D-91	1.5	1.7
D-95	3.8	2.7
D-97	3.6	2.4
E-39	3.1	2.9
Avg. ± S.D.	2.9 ± 0.9	2.1 ± 0.9
4-Day Exposures		
D-63	3.6	1.9
D-98	2.1	0.9
E-27	2.4	1.1
Avg. ± S.D.	2.7 ± 0.8	1.3 ± 0.5
7-Day Exposures		
E-31	3.6	1.6
E-35	2.7	1.5
E-37	2.3	1.1
Avg. ± S.D.	2.9 ± 0.7	1.4 ± 0.3
12-Day Exposures		
D-77	1.7	1.1
E-04	2.9	0.9
Avg. ± S.D.	2.3 ± 0.8	1.0 ± 0.1

The Q(O/N) values obtained with liver and kidney mitochondria using succinate and α-ketoglutarate substrates by the Warburg technique are shown in Table VI. The P/O ratios obtained using this method are shown in Table VII. A tendency toward lowered P/O values is evident with kidney mitochondria from exposed animals using both succinate and α-ketoglutarate. Inorganic phosphate esterified per mg nitrogen is shown in Table VIII. P/N values correlate with the product of the Q(O/N) and P/O values (although the determination of P/N values does not involve respirometric measurements) and may be considered as net production of ATP per mg mitochondrial nitrogen. This value, then, gives some idea of the activity of the mitochondria and of the resultant levels of tissue ATP to be expected if the numbers of mitochondria in the tissue are not decreased or ATP utilizing reactions are not proportionately elevated. Again, in general there appears to be a decrease in this parameter in the exposed animals.

Table IX shows the Q(O/N) values obtained in state 4 using the GME Oxygraph. Here the trend appears to be an elevation of rate with mitochondria from animals exposed to 100% oxygen at ambient pressure. A partial uncoupling of mitochondrial oxidative phosphorylation with resulting elevated ATP-ase activity would produce such a result. Some ADP would be continually regenerated by the ATP-ase reaction from added or endogenous ATP with resultant stimulation of oxidation.

Table X shows the Q(O/N) in state 3, i.e., mitochondrial respiration in the presence of excess ADP. There is no trend evident here of impaired mitochondrial function.

ADP/O ratios are shown in Table XI. In contrast to the P/O values observed using the Warburg respirometer, the ADP/O ratios obtained with mitochondria from animals exposed to 100% oxygen appear little changed. Although P/O values and ADP/O values are the analogous measurements in Warburg respirometer and GME oxygraph, they are not exactly comparable. The Warburg measurements were conducted at 30 C and those in the GME Oxygraph were conducted at 25 C -- preliminary experiments conducted at 30 C are not included in the results -- in addition, there was no ATP trapping system in the Oxygraph cell, i.e. glucose and hexokinase. Furthermore, incubation times were different: Warburg incubations involve 12 min preincubation and 10 min incubation of the mitochondria, while the length of one cycle in the Oxygraph incubations averaged 5 min for liver mitochondria using succinate substrate, 8 min for liver mitochondria using α-ketoglutarate, 4 min for kidney mitochondria using succinate, and 5 min for kidney mitochondria using α-ketoglutarate. Two cycles were run in succession, however, and the ADP/O values obtained in the successive cycles agreed quite well.

Table VI

Q(O/N) VALUES OF LIVER AND KIDNEY MITOCHONDRIA FROM MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

Monkey No.	Liver, (μl O_2/mg N/10 min)*		Kidney, (μl O_2/mg N/10 min)*	
	α-Ketoglutarate	Succinate	α-Ketoglutarate	Succinate
Ambient Controls				
D-85	13	67	74	98
D-91	26	72	105	101
D-95	35	62	106	115
D-96	49	33	75	64
D-97	39	68	116	132
Avg. $\pm$ S.D.	33 $\pm$ 14	61 $\pm$ 15	95 $\pm$ 20	102 $\pm$ 25
4-Day Exposure				
D-63	16	60	74	92
D-98	36	53	74	84
E-27	18	-**	52	-**
Avg. $\pm$ S.D.	23 $\pm$ 11	57 $\pm$ 5	67 $\pm$ 13	88 $\pm$ 5
7-Day Exposure				
E-31	42	67	65	74
E-35	29	69	93	107
E-37	17	63	66	83
Avg. $\pm$ S.D.	29 $\pm$ 13	66 $\pm$ 3	74 $\pm$ 16	88 $\pm$ 17
12-Day Exposure				
D-77	26	53	75	76
E-04	36	74	96	94
Avg. $\pm$ S.D.	31 $\pm$ 7	64 $\pm$ 15	85 $\pm$ 15	85 $\pm$ 12

*Obtained at 30 C using the Warburg Respirometer with α-Ketoglutarate or Succinate Substrates.

**Low oxygen uptake.

Table VII

P/O RATIOS OF LIVER AND KIDNEY MITOCHONDRIA FROM MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

Monkey No.	Liver P/O*		Kidney P/O*	
	α-Ketoglutarate	Succinate	α-Ketoglutarate	Succinate
Ambient Controls				
D-85	-	1.7	3.0	1.9
D-91	3.8	1.7	2.9	1.9
D-95	3.1	2.1	3.0	1.9
D-96	2.7	2.3	3.4	2.7
D-97	2.3	1.8	3.1	2.0
Avg. ± S.D.	3.0 ± 0.6	1.9 ± 0.3	3.1 ± 0.2	2.1 ± 0.3
4-Day Exposure				
D-63	3.4	1.5	2.7	1.7
D-98	2.6	1.5	2.2	1.7
E-27	3.1	-**	2.1	-**
Avg. ± S.D.	3.0 ± 0.4	1.5 ± 0.0	2.3 ± 0.3	1.7 ± 0.0
7-Day Exposure				
E-31	2.0	1.6	2.0	1.5
E-35	2.3	1.3	2.5	1.6
E-37	3.1	1.4	2.4	1.5
Avg. ± S.D.	2.5 ± 0.6	1.4 ± 0.2	2.3 ± 0.3	1.5 ± 0.1
12-Day Exposure				
D-77	2.4	1.5	2.5	1.6
E-04	2.6	1.4	2.8	1.9
Avg. ± S.D.	2.5 ± 0.2	1.4 ± 0.0	2.7 ± 0.2	1.8 ± 0.2

*Obtained at 30 C using the Warburg respirometer with α-Ketoglutarate or Succinate Substrates.

**Low oxygen uptake and consequent inaccurate P/O value.

Table VIII

INORGANIC PHOSPHATE ESTERIFIED PER MG MITOCHONDRIAL PROTEIN NITROGEN
(AT 30 C USING α-KETOGLUTARATE OR SUCCINATE SUBSTRATES)
BY LIVER AND KIDNEY MITOCHONDRIA FROM MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

	Liver (μmole P_i esterified/mg N/10 min)		Kidney (μmole P_i esterified/mg N/10 min)	
Monkey No.	α-Ketoglutarate	Succinate	α-Ketoglutarate	Succinate
Ambient Controls				
D-85	8.5	10.0	20.0	17.0
D-91	8.1	10.7	27.5	17.1
D-95	9.7	11.5	28.6	19.7
D-96	11.8	6.9	22.7	15.4
D-97	8.0	10.7	32.1	23.4
Avg. ± S.D.	9.2 ± 1.6	10.0 ± 1.8	26.2 ± 4.8	18.5 ± 3.1
4-Day Exposure				
D-63	4.5	7.9	17.9	14.0
D-98	8.4	6.9	14.6	12.6
E-27	5.3	--*	9.7	--*
Avg. ± S.D.	6.1 ± 2.0	7.5 ± 0.7	14.1 ± 4.1	13.3 ± 1.0
7-Day Exposure				
E-31	7.3	9.6	11.5	9.8
E-35	6.0	7.8	20.8	15.5
E-37	4.3	7.7	14.2	11.0
Avg. ± S.D.	5.9 ± 1.5	8.4 ± 1.1	15.5 ± 4.8	12.1 ± 3.0
12-Day Exposure				
D-77	5.4	7.0	16.9	10.9
E-04	8.4	9.3	24.1	16.0
Avg. ± S.D.	6.9 ± 2.1	8.1 ± 1.7	20.5 ± 5.1	13.5 ± 3.6

*Low respiration and consequent inaccurate P/N value.

Table IX

Q(O/N) IN STATE 4 OF LIVER AND KIDNEY MITOCHONDRIA FROM MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

Monkey No.	Liver Q(O/N) (μl O_2/mg N/10 min)*		Kidney Q(O/N) (μl O_2/mg N/10 min)*	
	α-Ketoglutarate	Succinate	α-Ketoglutarate	Succinate
Ambient Controls				
D-85	6.7	11.5	13.5	26.0
D-91	5.9	10.3	14.2	23.9
D-95	5.7	10.9	15.2	29.6
D-97	6.3	10.6	16.2	27.8
Avg. $\pm$ S.D.	6.2 $\pm$ 0.4	10.9 $\pm$ 0.5	14.8 $\pm$ 1.2	26.8 $\pm$ 2.5
4-Day Exposures				
D-63	6.4	11.2	10.5	20.7
D-98	7.8	13.4	16.1	27.2
E-27	7.7	13.6	14.4	25.2
Avg. $\pm$ S.D.	7.3 $\pm$ 0.8	12.7 $\pm$ 1.4	13.7 $\pm$ 2.9	24.4 $\pm$ 3.3
7-Day Exposures				
E-31	5.2	11.8	9.9	17.4
E-35	6.6	11.3	13.5	21.5
E-37	7.7	12.6	12.9	20.9
Avg. $\pm$ S.D.	6.5 $\pm$ 1.2	11.9 $\pm$ 0.7	12.1 $\pm$ 1.9	20.0 $\pm$ 2.2
12-Day Exposures				
D-77	8.4	14.7	22.4	37.3
E-04	6.7	12.5	15.7	26.4
Avg. $\pm$ S.D.	7.6 $\pm$ 1.2	13.6 $\pm$ 1.5	19.0 $\pm$ 4.8	31.9 $\pm$ 7.7

*Obtained at 25 C using a Clark Oxygen Electrode with α-Ketoglutarate or Succinate Substrates.

Table X

Q(O/N) IN STATE 3 OF LIVER AND KIDNEY MITOCHONDRIA FROM MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

Monkey No.	Liver Q(O/N) (μl O_2/mg N/10 min)*		Kidney Q(O/N) (μl O_2/mg N/10 min)*	
	α-Ketoglutarate	Succinate	α-Ketoglutarate	Succinate
Ambient Controls				
D-85	21.4	47.3	42.4	73.5
D-91	20.5	46.0	50.3	73.9
D-95	24.5	51.5	34.1	71.7
D-97	23.2	48.0	56.8	84.1
Avg. $\pm$ S.D.	22.4 $\pm$ 1.8	48.2 $\pm$ 2.4	45.9 $\pm$ 9.8	75.8 $\pm$ 5.6
4-Day Exposures				
D-63	19.8	48.5	44.1	84.9
D-98	23.0	45.2	35.4	53.6
E-27	21.0	51.2	28.8	60.4
Avg. $\pm$ S.D.	21.3 $\pm$ 1.6	48.3 $\pm$ 3.0	36.1 $\pm$ 7.6	66.3 $\pm$ 16.4
7-Day Exposures				
E-31	16.1	48.6	18.5	42.7
E-35	23.5	51.4	56.8	73.7
E-37	19.7	45.5	41.1	63.7
Avg. $\pm$ S.D.	19.8 $\pm$ 3.7	48.5 $\pm$ 3.0	38.8 $\pm$ 19.3	60.0 $\pm$ 15.8
12-Day Exposures				
D-77	22.3	43.1	54.2	75.7
E-04	22.6	54.8	54.2	82.1
Avg. $\pm$ S.D.	22.5 $\pm$ 0.2	48.9 $\pm$ 8.2	54.2 $\pm$ 0.1	78.9 $\pm$ 4.5

*Obtained at 25 C using a Clark Oxygen Electrode with α-Ketoglutarate or Succinate Substrates.

Table XI

ADP/O RATIOS (OBTAINED AT 25 C USING THE CLARK OXYGEN ELECTRODE WITH α-KETOGLUTARATE OR SUCCINATE SUBSTRATES) OF LIVER AND KIDNEY MITOCHONDRIA FROM MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

Monkey No.	Liver ADP/O		Kidney ADP/O	
	α-Ketoglutarate	Succinate	α-Ketoglutarate	Succinate
Ambient Controls				
D-85	2.0	1.5	2.0	1.4
D-91	2.2	1.5	2.1	1.5
D-95	2.1	1.5	1.9	1.3
D-97	2.1	1.5	2.1	1.5
Avg. ± S.D.	2.1 ± 0.1	1.5 ± 0.0	2.0 ± 0.1	1.4 ± 0.1
4-Day Exposures				
D-63	2.0	1.4	2.0	1.4
D-98	1.9	1.4	1.7	1.1
E-27	1.8	1.4	1.7	1.3
Avg. ± S.D.	1.9 ± 0.1	1.4 ± 0.0	1.8 ± 0.2	1.3 ± 0.2
7-Day Exposures				
E-31	1.9	1.5	1.7	1.3
E-35	2.0	1.4	2.1	1.4
E-37	1.9	1.3	2.1	1.4
Avg. ± S.D.	1.9 ± 0.1	1.4 ± 0.1	2.0 ± 0.2	1.4 ± 0.0
12-Day Exposures				
D-77	1.7	1.5	1.7	1.2
E-04	2.1	1.5	2.1	1.5
Avg. ± S.D.	1.9 ± 0.3	1.5 ± 0.0	1.9 ± 0.3	1.4 ± 0.2

RC values of liver and kidney mitochondria are shown in Table XII. These values are the average of the second and third cycles of duplicate incubations. In some cases, fewer cycles were obtained and the averages include less than four repeated cycles. Also, when the mitochondria had lost respiratory control, the resulting RC value of 1.0 was not included in the average shown in the table. Since the RC values of isolated mitochondria often tend to decrease upon standing, the first RC value has also been indicated in the table (i.e. the RC value observed in the second cycle of the first incubation). The average time lapse between duplicate incubations was 91 min, during which period, the mitochondrial suspensions were kept at 0 C. Essentially the same effects are seen whether average or initial RC values are compared. Some indication of the stability of the mitochondria can be obtained from comparing the average and initial RC values of a given mitochondrial suspension. No striking decrease in RC is seen for mitochondria isolated from the exposed animals, and this raises the question of why the P/O values were in general found to be somewhat lowered. Possible answers are that while the initial P/O ratios of mitochondria from exposed animals may be near normal values, the greater lability of these mitochondria in the incubation medium results in decreased oxidative phosphorylative efficiency or induces an elevated endogenous ATP-ase activity which then competes with the ATP trapping system in the medium. In such cases, the toxic effect of oxygen upon mitochondria, as reflected in these studies and in the morphological changes observed, may arise from some deleterious effect upon the stability of mitochondrial membranes.

EXPERIMENTS USING RATS AT 257 MM HG PRESSURE

Mitochondrial changes similar to but less severe than those seen with animal exposures to pure oxygen at ambient pressure are found with exposures to oxygen at 258 mm Hg pressure. In the case of Sprague Dawley rats, changes in renal and hepatic mitochondrial morphology were apparent after 3 day exposure. Changes were milder after 14 days exposure. Mitochondrial changes were very slight at 90 days, and all change had almost disappeared after 235 days of exposure (Klion, 1927; Mautner, 1966; Schaffner, 1965). Enlargement of mitochondria and mitochondria of bizarre shapes were seen after 3 days exposure. These changes in rats exposed 14 and 90 days were much milder and by 235 days had almost disappeared. Kaplan et al. (1968) studied rats after 235 days exposure to 100% oxygen at 258 mm Hg pressure and found no systemic toxicity. This study included P/O ratios, Q(O/N) values, and NAD/NADH ratios obtained by Riesen et al. P/O ratios obtained on Sprague Dawley rat liver mitochondria following exposures for 3, 4, 5 and 6 days in the Thomas dome at 5 psia revealed no significant decrease in P/O ratios using α-ketoglutarate substrate (Riesen, 1967). However, knowledge of the nature and extent of oxygen toxicity at nearly normal partial pressures is incomplete, especially for longer-term exposures (Davies, 1967).

Table XII

RC VALUES (OBTAINED AT 25 C USING A CLARK OXYGEN ELECTRODE WITH α-KETOGLUTARATE OR SUCCINATE SUBSTRATES) OF LIVER AND KIDNEY MITOCHONDRIA FROM MONKEYS EXPOSED TO 100% OXYGEN AT 748 MM HG

Monkey No.	Liver RC*		Kidney RC*	
	α-Ketoglutarate	Succinate	α-Ketoglutarate	Succinate
Ambient				
D-85	3.2 (3.2)	4.1 (4.3)	3.3 (5.5)	2.9 (3.5)
D-91	3.5 (3.4)	4.4 (4.5)	3.6 (3.8)	3.1 (3.4)
D-95	4.3 (4.4)	4.7 (4.9)	2.2 (3.0)	2.6 (3.0)
D-97	3.7 (3.5)	4.5 (5.2)	3.5 (3.8)	3.1 (3.4)
Avg. ± S.D.	3.7 ± 0.5 (3.6 ∓ 0.5)	4.5 ± 0.2 (4.7 ∓ 0.4)	3.1 ± 0.6 (4.0 ∓ 1.1)	2.9 ± 0.2 (3.3 ∓ 0.2)
4-Day Exposures				
D-63	3.1 (3.0)	4.4 (4.5)	4.2 (4.6)	4.1 (4.2)
D-98	2.9 (3.0)	3.4 (3.4)	2.2 (2.3)	2.0 (2.1)
E-27	2.7 (3.2)	3.8 (3.8)	2.0 (2.2)	2.4 (2.6)
Avg. ± S.D.	2.9 ± 0.2 (3.1 ∓ 0.1)	3.8 ± 0.5 (3.9 ∓ 0.5)	2.8 ± 1.2 (3.0 ∓ 1.3)	2.8 ± 1.1 (3.0 ∓ 1.1)
7-Day Exposures				
E-31	3.1 (2.9)	4.4 (3.9)	1.9 (2.3)	2.5 (2.9)
E-35	3.6 (3.6)	4.5 (4.5)	4.4 (5.1)	3.5 (3.7)
E-37	2.5 (2.5)	3.6 (3.4)	3.2 (3.4)	3.0 (3.2)
Avg. ± S.D.	3.1 ± 0.5 (3.0 ∓ 0.5)	4.1 ± 0.5 (3.9 ∓ 0.5)	3.1 ± 1.3 (3.6 ∓ 1.4)	3.0 ± 0.5 (3.3 ∓ 0.4)
12-Day Exposures				
D-77	2.7 (2.9)	2.9 (3.2)	2.4 (2.7)	2.1 (2.4)
E-04	3.4 (3.3)	4.4 (4.8)	3.5 (3.9)	3.1 (3.4)
Avg. ± S.D.	3.0 ± 0.5 (3.1 ∓ 0.2)	3.7 ± 1.0 (4.0 ∓ 1.1)	3.0 ± 0.7 (3.3 ∓ 0.8)	2.6 ± 0.7 (2.9 ∓ 0.7)

*Average values; initial RC values are shown in parentheses.

The present investigation involved exposures of 1, 4, 7, and 14 days during which time effects upon mitochondrial morphology are most pronounced. In addition, to determine the effect of return to ambient conditions after adaptation to one-third atmosphere oxygen, two groups of 14-day exposed rats were returned to ambient atmosphere and sacrificed 1 and 48 hr later.

Table XIII shows the ATP tissue concentrations of livers from rats after such exposures. There appears to be an initial elevation of tissue ATP levels at 257 mm Hg pressure which is especially noticeable at 4 days. Levels at 1 and 7 days also show significant elevations when compared with levels of concurrently-run control mitochondrial preparations. Under these conditions, mitochondria apparently can initially utilize the excess oxygen or are stimulated by its presence to produce additional ATP.

Tables XIV and XV show the average Q(O/N) values in state 4 obtained using α-ketoglutarate and succinate substrates. No striking elevations of oxidation rates are seen by mitochondria from exposed rats. However, in the case of succinate substrate, averages in the experimental groups at 1, 4, 7, and 14 days were higher than the ambient controls for these groups, but additional animals would be required to determine whether this elevation is statistically significant. Tables XVI and XVII show the average Q(O/N) values in state 3 using α-ketoglutarate and succinate substrates. In the case of succinate substrate, rates of the experimental groups at 1, 4, 7, and 14 days appeared elevated. For the 4-day group, this elevation was significant at the 10% level when values for mitochondria from paired animals were compared. No trends were apparent for the recovery experiments at the two times used.

ADP/O ratios are shown in Tables XVIII and XIX. Differences, if any, in the coupling of oxidation to phosphorylation caused by exposure to 100% oxygen at 257 mm Hg pressure, with or without subsequent recovery for 1 or 48 hr, are minimal. However, it is not known whether changes in P/O ratios would have been seen using the Warburg technique. In the experiments with monkeys exposed to 100% oxygen at ambient pressure, changes in P/O ratios obtained by the Warburg technique may be seen where little changes are obtained in ADP/O ratios using the GME Oxygraph.

RC values obtained for liver mitochondria from rats after various exposures to 100% oxygen at 257 mm Hg pressure are shown in Tables XX and XXI. Reflecting the relatively greater increase in state 3 oxidation rate compared to state 4 rate, the average RC value obtained (using succinate substrate) was elevated over the concurrently-run ambient controls for the 4-day exposed group. The difference was significant at the 1% level by the t test. Changes in the recovery groups were not statistically significant, except for RC values of mitochondria from rats exposed for

Table XIII

TISSUE ATP CONCENTRATIONS OF LIVERS FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

Exposure	ATP, μmoles/g* Experimental Average	Experimental S.D.	Ambient Controls Average	Ambient Controls S.D.	P(pooled)** <	P(paired)*** <
1 day	3.53	0.75 (10)	3.27	0.57 (11)	-	0.05
4 days	4.06	0.41 (10)	3.48	0.49 (11)	0.01[1]****	0.05[2]
7 days	3.60	0.59 (10)	3.10	0.39 (10)	0.05[3]	0.01
14 days	3.54	0.86 (10)	3.61	0.46 (9)	-	-
Mean			3.36	0.51 (41)		
14 days plus 1 hr recovery	3.49	0.58 (4)	3.52	0.34 (5)	-	-
14 days plus 48 hr recovery	3.25	0.48 (5)	3.38	0.26 (5)	-	-
Mean			3.45	0.29 (10)		

*Values for all animals except those which showed lung involvement on autopsy. P obtained using t test. Number of animals shown in brackets.

**Values for animals in one exposure period compared with pooled ambient controls obtained concurrently.

***Values for animals in one exposure period paired with concurrently-run controls.

****$P < 0.001$ when exposed animals in this group are compared to the overall average value of similarily treated ambient control animals for 1, 4, 7 and 14 days. If animals showing lung involvement are also included in this manner, $P < 0.01$.

[n]P calculated using all animals including those with lung involvement where a different significance value is obtained: 1. < 0.02; 2. < 0.02; 3, < 0.02.

Table XIV

Q(O/N) IN STATE 4 USING α-KETOGLUTARATE SUBSTRATE WITH LIVER MITOCHONDRIA FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

Exposure	Liver Q(O/N), μl O_2/mg N/10 min: Experimental Average	Experimental S.D.	Paired Controls Average	Paired Controls S.D.	No. of Pairs	P (paired) <
1 day	6.0	1.0	5.7	0.8	5	-
4 days	5.8	0.9	6.0	1.1	5	-
7 days	5.1	0.7	5.4	0.7	2*	x**
14 days	6.7	1.1	6.4	1.1	4	-
Recovery Experiments						
14 days plus 1 hr recovery	6.5	0.4	6.7	0.2	4	-
14 days plus 48 hr recovery	6.0	0.4	6.3	0.4	5	-

*Experimental average and S.D. includes one additional unpaired animal.

**P not significant by t test, using all animals including those with lung involvement (N = 5).

Table XV

Q(O/N) IN STATE 4 USING SUCCINATE SUBSTRATE OF LIVER MITOCHONDRIA FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

Exposure	Liver Q(O/N), μl O_2/mg N/10 min				No. of Pairs	P (paired) <
	Experimental		Ambient Controls			
	Average	S.D.	Average	S.D.		
1 day	13.4	2.2	11.9	2.6	5	-
4 days	13.9	2.6	12.1	1.0	5	-
7 days	13.5	3.4	13.2	0.6	2*	x**
14 days	16.4	3.7	13.9	3.1	4	0.10**
Recovery Experiments						
14 days plus 1 hr recovery	15.1	1.3	15.2	1.2	4	-
14 days plus 48 hr recovery	13.9	2.4	15.3	1.2	5	-

*Experimental average and S.D. includes one additional unpaired animal.

**P not significant by t test, using all animals including those with lung involvement (N = 5).

Table XVI

Q(O/N) IN STATE 3 USING α-KETOGLUTARATE SUBSTRATE OF LIVER MITOCHONDRIA FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

	Liver Q(O/N), μl O_2/mg N/10 min					
	Experimental		Paired Controls			
Exposure	Average	S.D.	Average	S.D.	No. of Pairs	P (paired) <
1 day	25.9	7.6	26.7	6.8	5	-
4 days	22.3	3.9	22.1	5.2	5	-
7 days	23.4	4.7	25.9	10.5	2*	x**
14 days	24.3	7.7	21.3	5.6	4	0.10**
	Recovery Experiments					
14 days plus 1 hr recovery	27.5	5.9	29.7	3.7	4	-
14 days plus 48 hr recovery	31.0	3.7	29.7	2.1	5	-

*Experimental average and S.D. includes one additional unpaired animal.

**P not significant by t test, using all animals including those with lung involvement (N = 5).

Table XVII

Q(O/N) IN STATE 3 USING SUCCINATE SUBSTRATE OF LIVER MITOCHONDRIA FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

	Liver Q(O/N), $\mu l\ O_2$/mg N/10 min					
	Experimental		Paired Controls			
Exposure	Average	S.D.	Average	S.D.	No. of Pairs	P (paired) <
1 day	72.2	10.6	61.3	13.3	5	-*
4 days	65.6	14.1	49.3	10.8	5	0.10
7 days	79.1	18.7	74.5	19.1	2**	x***
14 days	81.9	21.9	63.8	7.6	4	0.10***
	Recovery Experiments					
14 days plus 1 hr recovery	82.5	15.3	80.0	8.5	4	-
14 days plus 48 hr recovery	76.2	17.2	80.9	10.0	5	-

*P < 0.1 by t test using all animals including those with lung involvement (N = 6).

**Experimental average and S.D. includes one additional unpaired animal.

***P not significant by t test, using all animals including those with lung involvement (N = 5).

Table XVIII

ADP/O RATIOS OBTAINED USING α-KETOGLUTARATE SUBSTRATE OF LIVER MITOCHONDRIA FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

Exposure	ADP/O Experimental Average	Experimental S.D.	Ambient Controls Average	Ambient Controls S.D.	No. of Pairs	P (paired) <
1 day	2.34	0.08	2.35	0.11	5	-
4 days	2.23	0.23	2.19	0.09	5	-
7 days	2.35	0.09	2.41	0.21	2*	x
14 days	2.19	0.31	2.14	0.26	4	-
Recovery Experiments						
14 days plus 1 hr recovery	2.35	0.04	2.34	0.06	4	-
14 days plus 48 hr recovery	2.33	0.07	2.32	0.06	5	-

*Experimental average and S.D. includes one additional unpaired animal.

Table XIX

ADP/O RATIOS OBTAINED USING SUCCINATE SUBSTRATE OF LIVER MITOCHONDRIA FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

Exposure	ADP/O Experimental Average	ADP/O Experimental S.D.	ADP/O Ambient Controls Average	ADP/O Ambient Controls S.D.	No. of Pairs	P (paired) <
1 day	1.59	0.07	1.54	0.02	5	-
4 days	1.52	0.05	1.49	0.06	5	0.1
7 days	1.58	0.05[1]	1.58	0.07[2]	2*	x**
14 days	1.48	0.08	1.49	0.08	4	0.1
Recovery Experiments						
14 days plus 1 hr recovery	1.56	0.05	1.53	0.01	4	-
14 days plus 48 hr recovery	1.55	0.05	1.55	0.01	5	-

[n] ADP/O ± S.D. values obtained using all animals including those with lung involvement; (N = 5): 1. 1.54 ± 0.06, 2. 1.51 ± 0.07.

* Experimental average and S.D. includes one additional unpaired animal.

** $P < 0.05$ by t test using all animals including those with lung involvement (N = 5).

Table XX

RC VALUES OBTAINED USING α-KETOGLUTARATE SUBSTRATE OF LIVER MITOCHONDRIA FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

Exposure	RC Experimental Average	RC Experimental S.D.	RC Ambient Controls Average	RC Ambient Controls S.D.	No. of Pairs	P (paired) <
1 day	4.38	1.09	4.70	1.01	5	-
4 days	3.88	0.61	3.71	0.67	5	-
7 days	4.96	1.08	4.99	2.62	2*	x
14 days	3.71	1.22	3.47	1.19	4	-
Recovery Experiments						
14 days plus 1 hr recovery	4.26	0.66	4.44	0.64	4	-
14 days plus 48 hr recovery	5.22	0.50	4.54	0.50	5	0.05

*Experimental average and S.D. includes one additional unpaired animal.

Table XXI

RC VALUES OBTAINED USING SUCCINATE SUBSTRATE OF LIVER MITOCHONDRIA FROM RATS AFTER VARIOUS EXPOSURES TO 100% OXYGEN AT 257 MM HG

Exposure	RC Experimental Average	RC Experimental S.D.	RC Ambient Controls Average	RC Ambient Controls S.D.	No. of Pairs	P (paired) <
1 day	5.42	0.55	5.13	0.63	5	-
4 days	4.77	0.89	4.10	0.80	5	0.01
7 days	5.91	0.92	5.70	1.73	2*	x**
14 days	5.11	1.28	4.78	1.12	4	-
Recovery Experiments						
14 days plus 1 hr recovery	5.43	0.48	5.25	0.29	4	-
14 days plus 48 hr recovery	5.45	0.34	5.28	0.29	5	-

*Experimental average and S.D. includes one additional unpaired animal.

**P < 0.1 by t test, using all animals including those with lung involvement (N = 5).

14 days followed by 48 hr recovery using α-ketoglutarate substrate. The RC of this experimental group was elevated ($P < 0.05$) using five pairs of animals. Further experiments using larger groups of animals after recovery at these and other time intervals would be required to determine what changes occur during return to ambient conditions.

APPENDIX

STATISTICAL ANALYSIS OF DATA OBTAINED IN EXPERIMENTS USING MONKEYS AT 748 MM HG PRESSURE

To facilitate evaluation of results which were obtained in the investigation involving limited numbers of monkeys, a regression analysis of data was carried out by Dr. T. Peyser and Mr. A. Endres at IIT Research Institute using a BMD 02R computer routine. The objective of the analysis was to ascertain if duration of exposure to 100% oxygen at 748 mm Hg pressure had an effect on each of the sets of experimental measurements previously described in the text, and, if so, to obtain the line of best fit as a function of exposure time.

Multiple measurements on one mitochondrial preparation which were obtained using the Clark oxygen electrode (measurements were carried out on two cycles of duplicate incubations) were combined in the following manner for the regression analysis: an overall average was taken of the average values obtained during the second and third cycles of the first incubation and the average values obtained during the second and third cycles of the second incubation. For those cases in which both cycles were not obtained, the measurements obtained in the second cycle were considered equivalent to the average that would have been obtained with two cycles.

Regression equations of the form $y = c + b_1x + b_2x^2 + b_3x^3$ were obtained for the range of x values from 0 to 12 days (see Table XXII). The significance of the regression coefficients was tested by application of the F test. Only those terms in which the F values of the coefficients exceeded 3.0 were considered significant in their contribution to the regression analysis and included in this table (see Table XXIII). The constant terms (c) represent the values for the sets of experimental measurements (dependent variables, y) at $x = 0$, i.e., when ambient control animals were used. The regression equations for those sets of measurements for which significant coefficients were obtained can be written from the values of c, b_1, b_2 and b_3 listed in Table XXII. These equations can be used, within the time interval investigated, for estimation of the expected values for exposure times at which experiments were not performed. The form of these equations cannot be considered to be that of the actual mathematical functions which may relate duration of oxygen exposure to the values of the dependent variables.

The standard errors of the regression coefficients (b_1, b_2 and b_3) are shown in Table XXIV, and these values can be used by means of a t-table to set confidence limits on the regression coefficients at a specified probability level.

Table XXII

REGRESSION EQUATIONS: $y = c + b_1x + b_2x^2 + b_3x^3$, FOR THOSE PARAMETERS (DISCUSSED IN THE REPORT TEXT) FOR WHICH F VALUES OF THE REGRESSION COEFFICIENTS EXCEEDED 3.0

Dependent Variable* (y) ($0 \leq x \leq 12$ days)	c	b_1	b_2	b_3
Blood P_{O_2}** (IV)	91.38	282.947	-61.546	3.228
Blood P_{CO_2}** (IV)	26.99	2.752	-0.162	n.p.***
Kidney ATP (V)	1.994	-0.093	n.p.	n.p.
Q(O/N), Kidney, α-Ketoglutarate**** (VI)	94.27	-7.826	0.603	n.p.
P/O, Liver, Succinate (VII)	1.897	-0.123	0.007	n.p.
P/O, Kidney, α-Ketoglutarate (VII)	3.087	-0.239	0.0171	n.p.
P/O, Kidney, Succinate (VII)	2.026	-0.054	n.p.	n.p.
P/N, Liver, α-Ketoglutarate (VIII)	9.165	-0.964	0.065	n.p.
P/N, Kidney, α-Ketoglutarate (VIII)	25.87	-3.542	0.261	n.p.
P/N, Kidney, Succinate (VIII)	18.47	-1.651	0.103	n.p.
Q(O/N) State 4, Liver, Succinate*****(IX)	11.1	0.19	n.p.	n.p.
Q(O/N) State 4, Kidney, α-Ketoglutarate*****(IX)	14.92	n.p.	-0.168	0.016
Q(O/N) State 4, Kidney, Succinate*****(IX)	27.45	n.p.	-0.395	0.036
ADP/O, Liver, α-Ketoglutarate (XI)	2.06	-0.018	n.p.	n.p.
ADP/O, Liver, Succinate (XI)	1.46	-0.016	n.p.	0.00011
RC, Liver, α-Ketoglutarate****** (XII)	3.49	-0.055	n.p.	n.p.

*Number in parenthesis refers to Table in report concerned with this variable.

**Animal D-07 was not included in the regression analysis.

***"n.p." indicates that a term from this column is not present in the regression equation.

****Q(O/N) obtained using the Warburg respirometer.

*****Q(O/N) obtained in State 4 using the Clark oxygen electrode.

******A much more accurate regression equation can be obtained by employing a quadratic term although the F value for the quadratic coefficient (b_2) does not exceed 3.0. The c, b_1 and b_2 values for this equation are 3.64, -0.178 and 0.011, respectively.

Table XXIII

F VALUES OF REGRESSION COEFFICIENTS

Dependent Variable* (y) ($0 \leq x \leq 12$ days)	F Value of Coefficient b_1	b_2	b_3
Blood P_{O_2}** (IV)	11.41	9.59	8.54
Blood P_{CO_2} ** (IV)	7.55	3.65	-.-
Kidney ATP (V)	4.68	-.-	-.-
Q(O/N), Kidney, α-Ketoglutarate*** (VI)	5.60	4.49	-.-
P/O, Liver, Succinate (VII)	8.72	3.93	-.-
P/O, Kidney, α-Ketoglutarate (VII)	23.75	16.45	-.-
P/O, Kidney, Succinate (VII)	11.03	-.-	-.-
P/N, Liver, α-Ketoglutarate (VIII)	8.43	5.18	-.-
P/N, Kidney, α-Ketoglutarate (VIII)	14.46	10.63	-.-
P/N, Kidney, Succinate (VIII)	8.01	4.16	-.-
Q(O/N) State 4, Liver, Succinate**** (IX)	6.71	-.-	-.-
Q(O/N) State 4, Kidney, α-Ketoglutarate****(IX)	-.-	4.12	5.88
Q(O/N) State 4, Kidney, Succinate**** (IX)	-.-	9.58	11.81
ADP/O, Liver, α-Ketoglutarate (XI)	4.04	-.-	-.-
ADP/O, Liver, Succinate (XI)	6.30	-.-	6.51
RC, Liver, α-Ketoglutarate***** (XII)	3.15	-.-	-.-

*Number in parenthesis refers to Table in report concerned with this variable.

**Animal D-07 was not included in the regression analysis.

***Q(O/N) obtained using the Warburg respirometer.

****Q(O/N) obtained in State 4 using the Clark oxygen electrode.

*****For the quadratic regression equation (see Table XXII), the F values for the coefficients b_1 and b_2 are 3.88 and 2.06, respectively.

Table XXIV

STANDARD ERROR OF REGRESSION COEFFICIENTS

Dependent Variable* (y) ($0 < x < 12$ days)	Standard Error of Coefficient b_1	b_2	b_3
Blood P_{O_2} ** (IV)	83.757	19.873	1.105
Blood P_{CO_2} ** (IV)	1.001	0.085	-.-
Kidney ATP (V)	0.043	-.-	-.-
Q(O/N), Kidney, α-Ketoglutarate*** (VI)	3.306	0.284	-.-
P/O, Liver, Succinate (VII)	0.042	0.004	-.-
P/O, Kidney, α-Ketoglutarate (VII)	0.049	0.004	-.-
P/O, Kidney, Succinate (VII)	0.016	-.-	-.-
P/N, Liver, α-Ketoglutarate (VIII)	0.332	0.028	-.-
P/N, Kidney, α-Ketoglutarate (VIII)	0.931	0.080	-.-
P/N, Kidney, Succinate (VIII)	0.583	0.051	-.-
Q(O/N) State 4, Liver, Succinate****(IX)	0.074	-.-	-.-
Q(O/N) State 4, Kidney, α-Ketoglutarate****(IX)	-.-	0.083	0.0068
Q(O/N) State 4, Kidney, Succinate**** (IX)	-.-	0.128	0.0104
ADP/O, Liver, α-Ketoglutarate (XI)	0.0089	-.-	-.-
ADP/O, Liver, Succinate (XI)	0.0065	-.-	0.00004
RC, Liver, α-Ketoglutarate*****(XII)	0.031	-.-	-.-

*Number in parenthesis refers to Table in report concerned with this variable.

**Animal D-07 was not included in the regression analysis.

***Q(O/N) obtained using the Warburg respirometer.

****Q(O/N) obtained in State 4 using the Clark oxygen electrode.

*****For the quadratic regression equation (see Table XXII), the standard errors of the regression coefficients b_1 and b_2 are 0.090 and 0.0076, respectively.

Indices of the prediction accuracy of the regression equations, the Multiple Correlation Coefficients, are listed in Table XXV. The square of the Multiple Correlation Coefficient is the proportion of the variability of the dependent variable that is accounted for by the independent variable in the equation.

Table XXV also contains the F values for the overall significance of regression of the indicated dependent variable (i.e., the various sets of measurements described in the report text) on the independent variable, exposure time. The numbers in parentheses associated with the F values represent the degrees of freedom associated with the regression and residual variations. (The first number in the parentheses corresponds to the number of regression coefficients in the equation (N), and the second number represents the degree of freedom available for estimating residual variation. This latter number is equal to the number of reported experiments (E) minus the number of regression coefficients plus 1, i.e., E - (N + 1)).

The indicated significance levels, P, may be interpreted as an estimate of the probability of obtaining as large or larger an F statistic if the regressions were insignificant. Hence, in the sets of data obtained, those dependent variables with low P values appear to significantly change (not necessarily causually) with duration of exposure. The dependent variables with low P values are likely to be the parameters, of those investigated, which would most readily prove to be significantly affected by exposure to 100% oxygen at 748 mm Hg pressure.

Table XXV

MULTIPLE CORRELATION COEFFICIENTS, F VALUES AND ASSOCIATED SIGNIFICANCE LEVELS FOR REGRESSION EQUATIONS

Dependent Variable* (y) ($0 < x < 12$ days)	Multiple Correlation Coefficient	F Value For Regression Equation**	$P <$ For Regression Equation
Blood P_{O_2}*** (IV)	0.800	4.15 (3,7)	0.07
Blood P_{CO_2} *** (IV)	0.779	6.16 (2,8)	0.024
Kidney ATP (V)	0.546	4.68 (1,11)	0.07
Q(O/N), Kidney, α-Ketoglutarate**** (VI)	0.604	2.86 (2,10)	0.11
P/O, Liver, Succinate (VII)	0.795	7.73 (2,9)	0.015
P/O, Kidney, α-Ketoglutarate (VII)	0.852	13.26 (2,10)	0.005
P/O, Kidney, Succinate (VII)	0.724	11.04 (1,10)	0.008
P/N, Liver, α-Ketoglutarate (VIII)	0.715	5.22 (2,10)	0.03
P/N, Kidney, α-Ketoglutarate (VIII)	0.779	7.74 (2,10)	0.008
P/N, Kidney, Succinate (VIII)	0.756	6.10 (2,9)	0.02
Q(O/N) State 4, Liver, Succinate***** (IX)	0.6336	6.71 (1,10)	0.03
Q(O/N) State 4, Kidney, α-Ketoglutarate*****(IX)	0.7354	5.30 (2,9)	0.03
Q(O/N) State 4, Kidney, Succinate*****(IX)	0.7897	7.46 (2,9)	0.02
ADP/O, Liver, α-Ketoglutarate (XI)	0.5364	4.04 (1,10)	0.08
ADP/O, Liver, Succinate (XI)	0.6567	3.41 (2,9)	0.08
RC, Liver, α-Ketoglutarate******(XII)	0.489	3.15 (1,10)	0.12

*Number in parenthesis refers to Table in report concerned with this variable.

**Numbers in parentheses give the degrees of freedom associated with regression and residual variations.

***Animal D-07 was not included in the regression analysis.

****Q(O/N) obtained using the Warburg respirometer.

*****Q(O/N) obtained in State 4 using the Clark oxygen electrode.

******For the quadratic regression equation (see Table XXII), the Multiple Correlation Coefficient is 0.6173; the F value is 2.77 with 2 and 9 degrees of freedom associated with regression and residual variation; and the P value is less than 0.13.

REFERENCES

Chance, B., and Williams, G. R., 1956. The Respiratory Chain and Oxidative Phosphorylation, Adv. Enzymol. 17, 65-134.

Davies, R. E., 1967. Oxygen Toxicity at Near-Normal Partial Pressures, in Physiology in the Space Environment, Vol. II, Respiration, pp 93-98, National Academy of Sciences, National Research Council, Washington, D.C.

Haugaard, N., 1968. Cellular Mechanisms of Oxygen Toxicity, Physiol. Rev. 48, 311-373.

Kaplan, H. P., Thomas, A. A., Back, K. C., and Robinson, F. R., 1968. Evaluation of Animals Continuously Exposed to a 5 psia Pure Oxygen Space Cabin Atmosphere for Eight Months, Aerospace Med. 39, 63-67.

Klion, F. M., Schaffner, F., and Kaplan, H. P., 1967. Hepatic Effects of Breathing Pure Oxygen for Eight Months Upon Rats, Dogs and Monkeys, Aerospace Med. 38, 273-274.

Mautner, W., 1966. Electron Microscopic Investigations of Oxygen Effects on Kidney Tissue, in Proc. 2nd Annual Conf. Atmos. Contamination in Confined Spaces, May 4-5, 1966, pp. 170-175. AMRL-TR-66-120, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio.

Nelson, D. B., Highman, B., and Altland, P. D., 1967. Oxidative Phosphorylation During Altitude Acclimination in Rats, Am. J. Physiol. 213, 1414-1418.

Riesen, W. H., Kissane, J. Q., Bieber, R. W., Kardatzke, M. L., Kaplan, H. P., and Felig, P., 1966. Research on Oxygen Toxicity at the Cellular Level, AMRL-TR-206, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, iv + 76.

Riesen, W. H., Bieber, R. W., and Petersen, R. N., 1967. Research on Oxygen Toxicity at the Cellular Level, Final Technical Report, Contract No. AF 33(615)-5096, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, viii + 171.

Robinson, F. R., Harper, D. T., Kaplan, H. P., and Thomas, A. A., 1967. Pathology of Oxygen Toxicity in Forty Macaca Mulatta, AMRL-TR-66-234, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, iii + 16.

Schaffner, F., and Felig, P., 1965. Changes in Hepatic Structure in Rats Produced by Breathing Pure Oxygen, J. Cell Biol. 27, 505-517.

Thomas, A. A., 1965. Low Ambient Pressure Environments and Toxicity, Arch. Environ. Health 11, 316-322.

Weinbach, E. C., 1961. A Procedure for Isolating Stable Mitochondria from Rat Liver and Kidney, Anal. Biochem. 3, 335-343.